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(54) Title: IMMUNOSTIMULATORY OLIGONUCLEOTIDES WITH MODIFIED BASES AND METHODS OF USE  
THEREOF

(57) Abstract: Immunomodulatory oligonucleotide compositions are disclosed. These oligonucleotides comprise an immunostimulatory hexanucleotide sequence comprising a modified cytosine. These oligonucleotides can be administered in conjunction with an immunomodulatory peptide or antigen. Methods of modulating an immune response upon administration of the oligonucleotide comprising a modified immunostimulatory sequence are also disclosed.

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US 99/12538

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 6 C07H21/00 C07K2/00 C07K14/52 A61K31/70 A61K31/715 A61K38/00 A61K39/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07H C07K A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ, CHEM ABS Data, STRAND		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HARDIN, CHARLES C. ET AL: "Characterization of anti-Z-RNA polyclonal antibodies: epitope properties and recognition of Z-DNA" BIOCHEMISTRY (1988), 27(11), 4169-77, XP002129945 page 4169, right-hand column, last paragraph -page 4170, left-hand column, paragraph 3 page 4172, right-hand column, paragraph 2 -page 4173, left-hand column, paragraph 1 page 4174, left-hand column, paragraph 2 -right-hand column, paragraph 1 <div style="text-align: center;">-/-</div>	1-5, 24, 48
<div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.</span> <span><input checked="" type="checkbox"/> Patent family members are listed in annex.</span> </div>		
* Special categories of cited documents: <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>*Z* document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search	Date of mailing of the international search report	
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040. Tx. 31 651 epo nl. Fax (+31-70) 340-3016		Authorized officer  Held, P

# INTERNATIONAL SEARCH REPORT

In: International Application No  
PCT/US 99/12538

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		Relevant to claim No.
Category *	Citation of document, with indication, where appropriate, of the relevant passages	
X	BRENNAN, CATHERINE A. ET AL: "The effects of base analog substitutions on the methylation by the EcoRI modification methylase of octadeoxyribonucleotides containing modified EcoRI recognition sequences" J. BIOL. CHEM. (1986), 261(16), 7279-86, XP002143307 page 7279 page 7284, right-hand column, paragraph 3 see RN 103549-62-8	1,2,4,5
X	JEAN, YUCH CHENG ET AL: "Z-DNA structure of a modified DNA hexamer at 1,4-.ANG. resolution: aminohexyl-5'-d(pCpGp'Br5C!pGpCpG)" BIOCHEMISTRY (1993), 32(1), 381-8 , XP002143308 page 381 -page 382, left-hand column, paragraph 1 see RN 145586-04-5	1,2,4,5
X	NGUYEN H ET AL: "Studies Towards the Design of a Modified GC Base Pair With Stability Similar to that of the AT Base Pair" TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 38, no. 23, 9 June 1997 (1997-06-09), pages 4083-4086, XP004065032 ISSN: 0040-4039 the whole document	1,2,4,5
X	FERRER, ELISENDA ET AL: "Preparation and Properties of Oligodeoxynucleotides Containing 5-Iodouracil and 5-Bromo- and 5-Iodocytosine" BIOCONJUGATE CHEM. (1997), 8(5), 757-761 , XP002143309 page 757, right-hand column, paragraph 1 page 760, left-hand column see RN 194096-69-0	1,2,4,5
X	DUTTA, RATNA ET AL: "Binding of the modified daunorubicin WP401 adjacent to a T-G base pair induces the reverse Watson-Crick conformation: crystal structures of the WP401-TGGCCG and WP401-CGG'br5C!CG complexes" NUCLEIC ACIDS RES. (1998), 26(12), 3001-3005, XP002143310 page 3001 -page 3002, left-hand column see RN 211176-10-2	1,2,4,5

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International Application No

PCT/US 99/12538

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>THERIAULT, N. Y. ET AL: "Studies on the base pair binding specificity of CC-1065 to oligomer duplexes"  CHEM.-BIOL. INTERACT. (1988), 65(2), 187-201,  XP000929239  page 188 -page 189  table I, entry 10  see RN 116193-94-3</p>	1,2,4,5
X	<p>YOON, CHUN ET AL: "Structure of an alternating-B DNA helix and its relationship to A-tract DNA"  PROC. NATL. ACAD. SCI. U. S. A. (1988), 85(17), 6332-6,  XP002143311  page 6336, left-hand column, paragraph 1  figure 6  see RN 117978-22-0</p>	1,2,4,5
A	<p>KRIEG A M ET AL: "CPG MOTIFS IN BACTERIAL DNA TRIGGER DIRECT B-CELL ACTIVATION"  NATURE,GB,MACMILLAN JOURNALS LTD. LONDON, vol. 374, 6 April 1995 (1995-04-06), pages 546-549, XP000197060  ISSN: 0028-0836  page 546, right-hand column, paragraph 2 - paragraph 3  page 549, left-hand column, paragraph 1  table 1</p>	1,40
A	<p>GODDARD, AMANDA J. ET AL: "Synthesis of a phosphoramidite of 2'-deoxy-5,6-dihydro-5-azacytidine. Its potential application in the synthesis of DNA containing dihydro-5-aza an 5-azacytosine bases"  TETRAHEDRON LETT. (1988), 29(15), 1767-70,  XP002129946  page 1767, paragraph 1  page 1770</p>	1,40
A	<p>WO 89 09779 A (US GOVERNMENT)  19 October 1989 (1989-10-19)  page 1, line 4 - line 9  scheme 1</p>	1,40
A	<p>US 4 948 882 A (RUTH JERRY L)  14 August 1990 (1990-08-14)  column 4, line 22 - line 58  column 7</p>	1,40

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# INTERNATIONAL SEARCH REPORT

In: International Application No

PCT/US 99/12538

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>WO 98 55495 A (DYNAVAX TECHNOLOGIES CORP ;DINA DINO (US); ROMAN MARK (US); SCHWAR) 10 December 1998 (1998-12-10)</p> <p>page 1, line 10 - line 16 claims SEQ ID No 12, 15 and 16 -----</p>	<p>1,2,4-7, 9,11,12, 14,17, 18,20, 23-48</p>

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 99/12538

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 40-48 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
  
1-4 (partially), 5, 6-8 (partially), 9, 10, 11-13 (partially), 14-16, 17-19 (partially), 20-22, 23-28 (partially), 29-31, 32-48 (partially)
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1 (partially), 4 (partially), 6 (partially),  
11 (partially), 17 (partially), 23-28 (partially),  
32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the modified cytosine is an azacytosine, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

2. Claims: 1 (partially), 4 (partially), 6 (partially),  
11 (partially), 17 (partially), 23-28 (partially),  
32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is an azapyrimidine (not an azacytosine), in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

3. Claims: 1-4 (partially), 5, 6-8 (partially), 9, 10,  
11-13 (partially), 14-16, 17-19 (partially),  
20-22, 23-28 (partially), 29-31, 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the modified cytosine is 5-bromocytosine, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

4. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is bromouracil, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

oligonucleotide as well as the use of such a composition to modulate an immune response

5. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a bromopyrimidine (not a cytosine or an uracil), in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

6. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a chloropyrimidine, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

7. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a fluoropyrimidine, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

8. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a 5-trifluoromethylpyrimidine, in so far as it is neither a



## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

9. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a 5,6-dihydropyrimidine, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

10. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a iodopyrimidine, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

11. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a nitropyrimidine, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

12. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

hydroxyurea, in so far as it is neither a cyclocytosine nor a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

13. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the modified cytosine is a cyclocytosine; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

14. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the modified cytosine is a cytosine arabinoside; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

15. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially), 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is uracil; a composition containing such an oligonucleotide as well as the use of such a composition to modulate an immune response

16. Claims: 1-4 (partially), 6-8 (partially),  
11-13 (partially), 17-19 (partially),  
23-28 (partially); 32-48 (partially)

An immunomodulatory oligonucleotide comprising an immunostimulatory sequence comprising a modified cytosine characterized in that the "modified cytosine" is a pyrimidine not belonging to the groups of compounds mentioned in subjects 1-15; composition containing such an oligonucleotide as well as the use of such a composition to

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

modulate an immune response

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/12538

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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